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Biomass production and energy balance of herbaceous and woody crops on marginal soils in the Po Valley

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Abstract

A wealth of data and information on the cultivation of perennial biomass crops has been collected, but direct comparisons between herbaceous and woody crops are rare. The main objective of this research was to compare the biomass yield, the energy balance and the biomass quality of six perennial bioenergy crops: Populus spp., Robinia pseudoacacia, Salix spp., Arundo donax, Miscanthus × giganteus, and Panicum virgatum, grown in two marginal environments. For giant reed and switchgrass, two levels of nitrogen fertilization were applied annually (0–100 kg ha^{-1}). Nitrogen fertilization did not affect biomass or energy production of giant reed; thus, it significantly reduced the energy return on investment (EROI) (from 73 to 27). In switchgrass, nitrogen fertilization significantly increased biomass production and the capacity of this crop to respond to water availability, making it a favorable option when only biomass production is a target. Net energy gain (NEG) was higher for herbaceous crops than for woody crops. In Casale, EROI calculated for poplar and willow (7, on average) was significantly lower than that of the other crops (14, on average). In Gariga, the highest EROI was calculated for miscanthus (98), followed by nonfertilized giant reed and switchgrass (82 and 73, respectively). Growing degree days$_{10}$ during the cropping season had no effect on biomass production in any of the studied species, although water availability from May to August was a major factor affecting biomass yield in herbaceous crops. Overall, herbaceous crops had the highest ranking for bioenergy production due to their high biomass yield, high net energy gain (NEG), and biomass quality that renders them suitable to both biochemical and thermochemical conversion. Miscanthus in particular had the highest EROI in both locations (16 and 98, in Casale and Gariga), while giant reed had the highest NEG on the silty-loam soil of Gariga.

Keywords: *Arundo donax*, energy balance, *Miscanthus × giganteus*, nitrogen, *Panicum virgatum*, *Populus* spp., quality, *Robinia pseudoacacia*, *Salix* spp yield

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Introduction

Cultivation of perennial bioenergy crops is an important option in meeting future global energy demand (Creutzig *et al.*, 2015). Over the last decade, the possibility of cultivating bioenergy crops on marginal land, unsuitable for food production, has been proposed (Dauber *et al.*, 2012) as a possible solution to the so-called 'food, energy, and environment trilemma' (Tilman *et al.*, 2009).

A wealth of data and information on the cultivation of perennial biomass crops has been collected in recent years, but direct comparisons between herbaceous and woody crops are rare on marginal soils. Field experiments designed to directly compare the cultivation of herbaceous and woody bioenergy crops enable the ranking of the same crops according to a list of potential agronomic, economic, and environmental aspects (Table 1).

In the last years, it was shown that the cultivation of perennial bioenergy crops combines the supply of biomass for renewable energy production with a general increase in the provision of multiple key ecosystem services (Milner *et al.*, 2015). Positive impacts on the provision of ecosystem services were strictly dependent on the type of land use replaced (Holland *et al.*, 2015) and on the spatial allocation of the crops relative to the adjacent land uses (Werling *et al.*, 2013; Bourke *et al.*, 2014). The integration of perennial crops into agricultural landscapes could also promote the mitigation of ecosystem disservices from annual food cropping systems, as revealed in several studies (Powers *et al.*, 2011; Parish *et al.*, 2012; Meehan *et al.*, 2013).

Herbaceous and woody crops are considered promising carbon-neutral options because of their long-term

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Table 1 Crop ranking based on the main agronomic, environmental, and economic advantages of the cultivation of the herbaceous and woody crops. Only review papers that dealt with at least one herbaceous and one woody crop were considered

	Herbaceous crops			Woody crops		
	Giant reed	Switchgrass	Miscanthus	Poplar	Willow	Black locust
Genotype availability	+	++	+	+++	+++	+++
Crop management*	++†	++†	++†	+++†	+++†	?
High yielding	+++‡	++‡	+++‡	++‡	++‡	?
Multipurpose	++	++	++	+	+	+
Nutrient use efficiency	+++†	+++†	+++†	+++†	+++†	+++†
Drought resistance	+§	++/+++§	++§	!!§	!!§	+++§
Flood tolerance	++§	++§	++§	!!§	!!§	+§
Heat tolerance	+++§	++§	++§	!!§	!!§	++§
Energy balance	+++¶	+++¶	+++¶	++¶	++¶	++¶
Soil C sequestration	+++**	+++**	+++**	++**	++**	++**
Biodiversity	++††	++††	++††	++††	++††	++††
Water quality	+++‡‡	+++‡‡	+++‡‡	+++‡‡	+++‡‡	+++‡‡
Invasiveness	+++†	+†	+†	+†	+†	?
Economic life span	+†	+++†	+++†	++†	++†	++†

The symbols '+', '++', and '+++' indicate low, moderate, and high advantage, respectively; '?' stands for not available information, while '!!' varies on the basis of the different crops and genotype.
*The ranking is based on the status of the current farming, harvesting, and processing technologies (Zegada-Lizarazu *et al.*, 2010).
†Zegada-Lizarazu *et al.* (2010).
‡Laurent *et al.* (2015).
§Quinn *et al.* (2015).
¶Rettenmaier *et al.* (2010).
**Agostini *et al.* (2015).
††Dauber *et al.* (2010) (reported only the impact at field scale).
‡‡Ssegane *et al.* (2015).

soil C storage potential (Agostini *et al.*, 2015; Chimento *et al.*, 2016). Perennial herbaceous (Werling *et al.*, 2013) and woody crops (Rowe *et al.*, 2013) can also sustain a variety of ecosystem functions (such as pest suppression and pollination), promoting the creation of multifunctional agricultural landscapes. Moreover, reduced N losses (Smith *et al.*, 2013), reduced soil erosion (Kort *et al.*, 1998), nutrients removal from runoff (Lee *et al.*, 2003), and N removal from groundwater (Ssegane *et al.*, 2015) have been reported for herbaceous crops, such as *Miscanthus* × *giganteus* L. and *Panicum virgatum* L. In general, sustainability of biomass production can be achieved by cultivating high-yielding low-input crops (Ercoli *et al.*, 1999) on marginal soils (Powlson *et al.*, 2011).

Availability of genetic material, tested in different pedoclimatic conditions, is relatively large for woody crops (Cunniff *et al.*, 2015) and ongoing breeding activities will further extend farmer options (Hallingbäck *et al.*, 2015). Several *Populus* spp (Dillen *et al.*, 2013; Verlinden *et al.*, 2013) and *Salix* spp clones (Rosso *et al.*, 2013; Amichev *et al.*, 2014) are available to be grown on marginal soils in short-rotation coppice. In the case of perennial herbaceous crops, breeding of *Panicum virgatum* and *Miscanthus* spp is ongoing (Liu *et al.*, 2015; Tamura *et al.*, 2015), but the actual selection of genotypes is limited to a number of American switchgrass varieties and to a single genotype of *Miscanthus* × *giganteus* L. (Zegada-Lizarazu *et al.*, 2010). No breeding has been performed on giant reed and available field data are relative to local clones that have shown very limited genetic (Ahmad *et al.*, 2008) or phenotypic diversity (Amaducci & Perego, 2015).

Crop management is well established for woody crops and switchgrass, while some adjustments are need for miscanthus and especially for *A. donax*. Reduction of establishment costs in *Miscanthus* × *giganteus* is pursued via the selection of fertile genotypes that can be sown (Anderson *et al.*, 2015; Xue *et al.*, 2015), while this does not seem an option for *A. donax* (Pilu *et al.*, 2013). Mechanization and storage of giant reed is still an open issue (Bentini & Martelli, 2013; Pari *et al.*, 2015).

Biomass yield is one of the most relevant parameters to assess biomass crops performance, but it is strongly depended on environmental conditions and direct comparisons are needed to identify the most suitable crops for a specific condition. In general, it is reported that

perennial herbaceous crops have a greater biomass production compared to woody crops (Nassi o Di Nasso *et al*., 2010; Rettenmaier *et al*., 2010; Laurent *et al*., 2015). As biomass yield has a significant impact on bioenergy yield and on greenhouse gas (GHG) savings, herbaceous crops seem to have a better environmental impact than woody crops (Rettenmaier *et al*., 2010; Creutzig *et al*., 2015).

The environmental and productive performance of a crop is well depicted by its efficiency in using nitrogen and water. It is reported nitrogen fertilization is necessary to support high biomass production in woody crops (Heilman & Norby, 1998; Kauter *et al*., 2003), while the effect of nitrogen is limited or not significant (Heaton *et al*., 2004a) in herbaceous crops. The deeper root system of perennial herbaceous crops compared to woody crops (Chimento & Amaducci, 2015) can explain the higher productivity of herbaceous crops in water-limited conditions (Monti & Zatta, 2009a).

Relatively to biomass quality, low lignin content and high digestibility render herbaceous biomass crops suitable for second-generation biofuel production (Monti *et al*., 2015), while energy application of woody crops is generally limited to thermochemical conversion (Demirbas, 2004).

Previous work on biomass production on marginal lands has been based primarily on the landscape's suitability (Gopalakrishnan *et al*., 2011; Harvolk *et al*., 2014), while research to directly compare the performance of herbaceous and woody crops is very limited. Direct comparisons are useful to reliably characterize different biomass crops for their yield potential (Laurent *et al*., 2015). More comparative multispecies field trials and monitoring are needed within a range of climatic and soil conditions to fully understand the energy efficiency of various bioenergy cropping systems. Resolving the crop ranking from direct comparison of herbaceous and woody crops is critical to identify and promote the cropping systems able to provide the greatest energy efficiency for a given marginal environment.

In this study, the main objective was to compare the biomass yield, the energy balance, and the biomass quality of six perennial bioenergy crops: three woody crops *Populus* spp. (poplar), *Robinia pseudoacacia* (black locust), and *Salix* spp. (willow) – and three herbaceous crops – *Arundo donax* (giant reed), *Miscanthus* × *giganteus* (miscanthus), and *Panicum virgatum* (switchgrass), grown in two marginal environments within the agricultural landscape of the Po Valley (northern Italy), where two multispecies field trials ('Casale' and 'Gariga') were set up. The Gariga trial has been already investigated for establishing the crop ranking in soil C storage (Chimento *et al*., 2016) and belowground biomass (Chimento & Amaducci, 2015).

Materials and methods

Study site and experimental design

Two field trials were established in April 2007 in the Po Valley, the first at Gariga di Podenzano, Italy (44°58′48″N, 9°41′09″E), on a silt loam soil classified as chromic luvisols (FAO) with low carbonate content and neutral pH and the second at Casale Monferrato, Italy (45°08′57′′N, 8°30′56′′E), on a sandy soil, classified as fluvisol. Prior to planting, the experimental site had hosted a maize monoculture for 30 years in Gariga, and in Casale, the site hosted poplar stands for about 30 years and set aside for the last five years prior to the experiment. Both locations are to be regarded as marginal land for their soil quality and position within the agricultural landscape. The soil in Casale has an extremely high content of sand (>90%) and it is on the flood plain of the Po River; in Gariga, the soil is compacted, silty with a low content of organic carbon (8 g kg^{-1} soil), and the experimental site is located along a main road where sprinkle irrigation is not possible. Soil characteristics are presented in Table S1.

At both sites, the experimental layout is a randomized complete block design with three blocks and a single plot size of 450 m^2 (15 × 30 m), to compare six biomass crops; three herbaceous, giant reed (*A. donax L.*), switchgrass (*P. virgatum*), and miscanthus (*Miscanthus* × *giganteus*), and three woody bioenergy crops, poplar (*Populus* spp), willow (*Salix* spp), and black locust (*R. pseudoacacia*). The plots of the woody crops were split in four subplots, each hosting a different clone, so that for each woody crop, the same four clones were compared at each location. Data relative to the comparison among clones will not be presented in this study, and the biomass yield data of the most productive clones for each crop in each location will be used for the comparison among crops. The most productive clones were *Baldo* (*Populus deltoides*) and *Orion* (*Populus x canadensis*), *S76-008* and *Levante* (hybrids of *Salix babylonica* L.), in Casale and Gariga, respectively, while *Calabria* (ecotype from southern Italy) was the most productive black locust provenance at both sites.

At both locations, planting was carried out after typical soil preparation (30 cm deep ploughing followed by 2 passages of rotary tiller), using identical planting densities and propagating material. Giant reed was planted using portion of rhizomes (on average 300 g each) from a local ecotype (the same close at each location), at 1 rhizome per m^{-2}. Miscanthus was planted with rhizomes (on average 50 g each) imported from UK (ADAS Ltd, Ely, Cambridgeshire) at 1.5 rhizomes per m^{-2}. Switchgrass, var Alamo, was sown with an experimental mechanical seed drill (Vignoli) using 0.25 g of pure live seeds (pls) per m^{-2}. Inter-row distance was 1.4 m in giant reed and miscanthus and 0.4 m in switchgrass.

Clones of poplar and willow and provenances of black locust were provided by CREA-PLF (Casale Monferrato – Italy). Stem cuttings (for poplar and willow) and 1-year seedlings (for back locust) were transplanted manually in rows with an interplant distance of 0.6 m and an inter-row distance of 2.5 m (0.67 plants m^{-2}).

For all crops, biomass yield was estimated by weighing all the plants harvested on an area of approximately 10 m^2 in each

plot. Dry matter content was estimated gravimetrically on a biomass subsample (approximately 1 kg) weighted at harvest and after 24 h at 105 °C.

To assess the effect of environmental factors on biomass production of the herbaceous crops, growing degree $days_{10}$ and water input were estimated from June to September of each year in both locations in agreement with Triana *et al.* (2015), who recently found in a 2-year lysimeter experiment in central Italy that giant reed and miscanthus had the highest water requirements from June to September. Growing degree $days_{10}$ were calculated for each year by summing the daily difference between mean air temperature and the base temperature, which is 10 °C for miscanthus and switchgrass (Arundale *et al.*, 2015); the same value of base temperature was assumed for giant reed that is regarded as a macrothermal crop (Cappelli *et al.*, 2015). When daily mean temperature was lower than 10 °C, then growing degree day_{10} was null. A linear regression was performed to test the response of herbaceous biomass production to the water input (irrigation + rainfall) calculated from June to September. As the regression was found to be not significant ($r^2 = 0.01$) for all the crops in both locations, the regression was then executed on biomass production and the water input calculated from May to August because May rainfall variability between years was higher than that of September (coefficient of variation = 75% and 49%, respectively). The linear regression was executed between herbaceous biomass production and growing degree $days_{10}$ from May to August.

In addition to the abovementioned common features of the experimental design, there were some management factors that differed between the two sites. These are listed as follows.

In Casale, from 2007 to 2012, two irrigations were applied annually to both herbaceous and woody crops from late May to early August according to the weather conditions. The annual amount of irrigation water was 70 mm (35 mm of water per irrigation event).

In Gariga, no irrigation was applied.

In Gariga, nitrogen fertilization was carried out on a selection of plots at a rate of 100 kg ha^{-1} of nitrogen using ammonium nitrate. Nitrogen was applied (i) to the whole plot of woody crops at the beginning of growth in the spring after the harvest (in 2009, 2011, 2014), and (ii) to one half of each plot of giant reed and switchgrass at the beginning of growth in the spring after the harvest (every year excluding 2007, the year of establishment) in order to study the response of biomass production to nitrogen fertilization. In Gariga, the effect of nitrogen fertilization was assessed only on giant reed and switchgrass because the plant stand for these crops was uniform and it was possible to split the plot into two subplots to apply the nitrogen and no-nitrogen treatment; miscanthus plant establishment was not uniform enough for the same treatment to be applied.

In Casale, nitrogen fertilization was never provided to any crop in order to simulate a condition of low-input cropping.

Harvesting was carried out every year at the end of winter for the herbaceous crops, with the exception of Gariga where miscanthus was not harvested at the end of the first year due to the very limited biomass production (estimated <1 Mg ha^{-1}).

In Casale, woody crops were harvested at the end of year 2, 4, and 6.

In Gariga, woody crops were harvested at the end of year 2, 4, and 7. It was decided to postpone the third harvesting to year 7 for the limited plant growth achieved as a consequence of the extreme drought of 2012.

In Gariga, to compare qualitative characteristics of herbaceous and woody crops, cellulose, hemicellulose, lignin, and ash content were determined on biomass samples collected at harvesting in 2013. The analysis was carried out using the AnkomII Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA) and was corrected for the residual ash content, following the procedure described in Gallo *et al.* (2013).

Energy balance: inputs and outputs determination

The energy balance for biomass production was calculated using the data collected during the field trials in Casale. For each crop, the energy potentially delivered by combustion of lignocellulosic material (gross energy yield) was estimated considering the dry aboveground biomass production and its lower heating value, LHV (McKendry, 2002). The LHV for each crop was measured by IKA C200 calorimeter at CREA-PLF of Casale Monferrato (Alessandria, northern Italy). The energy required for crop establishment (soil preparation and planting), cultivation (control of weeds, fertilization and irrigation), and harvest of lignocellulosic material was calculated considering number of operations, time required per operation (h ha^{-1}), type of machines, relative power (kW), and diesel oil consumption (l h^{-1}).

The analysis accounted for direct and indirect energy costs (Hülsbergen *et al.*, 2001): ‘Direct costs’ included diesel oil consumed for each operation, while the consumption of lubricants was neglected. It was assumed that 1 l of diesel oil contains 35.9 MJ (Dalgaard *et al.*, 2001). The ‘indirect costs’ were related to the manufacture of fertilizers, pesticides and herbicides, machines and equipment, and propagation material for planting. Production of fertilizers requires a very high energetic cost, especially nitrogen fertilizers: We assumed an energetic cost of 73.3, 13.4, and 9.2 MJ kg^{-1} for the production of nitrogen, phosphorus, and potassium fertilizers, respectively (Manzone & Calvo, 2016). The production of other chemical compounds like pesticides and herbicides requires, respectively, 53 and 91 MJ kg^{-1} (Green & McCulloch, 1976; Green *et al.*, 1987). The energetic costs for the construction of machines were derived from Fiala & Bacenetti (2012): 92 MJ kg^{-1} in case of tractor and forager and 69 MJ kg^{-1} in case of other equipment (e.g., header for harvest). These values were then divided by the life span (i.e., 800 h per year) and the effective annual operation time per hectare. In this analysis, life span of the machines was assumed to be 10 years.

It was considered that, for sprinkle irrigation delivering about 350 m^3 ha^{-1} of water, an engine of 100 kW works for 6 h to pump water from a five meters deep well and consumes 74 l of diesel fuel, corresponding to 2657 MJ (Lal, 2004). The energetic cost relative to propagating material was 0.3 MJ $tree^{-1}$ (Dillen *et al.*, 2013) for the woody crops (i.e., cutting of poplar and willow or one-year-old seedlings of black

locust). Energy costs for propagating material in herbaceous crops are usually considered as negligible (Lettens *et al.*, 2003; Angelini *et al.*, 2005); in this work, we assumed an energy cost of 44 MJ kg^{-1} for producing switchgrass seeds (Schmer *et al.*, 2008) and an energy cost of 0.2 and 0.15 MJ per rhizome for giant reed and miscanthus, respectively (Ecoinvent, 2014).

The total costs of woody crops harvesting varied according to annual biomass production: An unit energy cost of 0.23 GJ t^{-1} of dry matter was assumed for woody crops collected by self-propelled combine forager harvester (Claas Jaguar 880) equipped with the header GBE_2 (Fiala & Bacenetti, 2012). The same cost was assumed for the harvesting of miscanthus and giant reed that can be carried out with a forage harvester. Switchgrass was harvested by shredding and baling with a direct cost of 5 l of diesel oil per hour as measured in Casale. Time requested for harvest varied on the basis of biomass production.

Table S2 reports energy costs of planting, management, and harvesting (direct and indirect costs) for each crop in both locations.

The estimation of energy costs (input) and gross energy yield (output) enable the net energy gain (input–output, NEG) and the energy return on investment (EROI) index to be calculated for both herbaceous and woody crops. NEG is an energy metric and is defined as the gained difference in energy between the energy content of the biomass at the farm gate and the total energy invested to produce it (Hill *et al.*, 2006).

EROI is dimensionless and quantifies the efficiency of different energy technologies and it is the ratio between the amount of energy produced (expected return) and the nonrenewable primary energy needed to produce it (investment) (Hall *et al.*, 2009).

Statistical analysis

Crops, year, and location effects on biomass production were tested using a repeated-measures mixed model in a randomized complete block design using IBM – SPSS 21 (IBM Corporation, Armonk, New York, US). Crop, year, and location were included in the design as fixed factors; year was also specified as the repeated-measures term. Year was taken as a fixed factor as it represented the stand age of the perennial crops. First, the mixed model was applied solely to data of herbaceous crops collected from annual harvests from 2007 to 2014. Then, the model was run on the biomass data collected for the woody crops at three harvesting times: in 2008, 2010, and 2012 in Casale and in 2008, 2010, and 2013 in Gariga.

The mixed model was used to examine differences in biomass production between woody and herbaceous crops. With this regard, annual biomass production was estimated for each woody crop dividing by two the production of a single harvest, with the exception of the third harvest in Gariga that was divided by three to estimate the average annual production from 2011 to 2013. As the effect of location overwhelmed the crop effect on both herbaceous and woody crops production, the mixed model was performed for the two locations independently.

A repeated-measures mixed model was applied to the data of giant reed and switchgrass collected under two levels of nitrogen fertilization from 2008 to 2014 in Gariga. As crop overwhelmed the effect of nitrogen fertilization, the mixed model was performed on giant reed and switchgrass independently.

A two-way ANOVA model was applied to data of NEG and EROI index estimated over the whole experimental period in order to find differences between crops and locations.

A one-way ANOVA was run to assess changes in cellulose, hemicellulose, lignin, and ash content between crops using data collected in 2013 in Gariga.

Comparison of means was performed by *post hoc* Tukey's HSD test (Tukey, 1953) when main effects or interaction of factors were found significant according to the mixed model or to the univariate ANOVA.

A linear regression analysis was carried out to study the relationship between biomass production and environmental factors, namely growing degree $days_{10}$ and water input.

Results

In the present study, that reports on the first eight years (2007–2014) after establishment of six perennial bioenergy crops, a wide range of rainfall variability was experienced in the two field trials (Table S3). The monthly distribution of rainfall in spring and summer varied between years. In particular, a wide range of rainfall was encountered both in Casale and in Gariga in May (13–173 and 3–158 mm, respectively), in June (7–153 and 11–150 mm, respectively), in July (3–106 and 0–76 mm, respectively), and in August (8–139 mm and 0–68 mm, respectively). The mean coefficient of variation of monthly rainfall from May to August across years was 73% and 86% in Casale and in Gariga, respectively. Annual rainfall ranged from 534 mm (2007) to 1053 mm (2014) in Casale, and 548 mm (2012) from to 1070 mm (2010) in Gariga; the mean annual rainfall was 818 and 757 mm in Casale and in Gariga, respectively.

Biomass production

When using the mixed model to examine the effects of herbaceous crops, location, and year, biomass production was significantly higher in giant reed and miscanthus (14.8 and 13.3 Mg ha^{-1} yr^{-1}, respectively) than in switchgrass (10.1 Mg ha^{-1} yr^{-1}) across years and locations (Table 2A). As the two-way interaction between years and locations was significant, each location was analyzed independently. In Casale, biomass production varied significantly between crops and years (Table 2B). On average, biomass yield measured at the year of establishment was lower than that achieved in subsequent years, considering that after the second year no biomass difference was found between years, it can be assumed that all herbaceous crops reached their maxi-

Table 2 (A) Mixed-model analysis of variance of the fixed effects of crop, year, and location on data of herbaceous dry biomass production collected in Casale and in Gariga from 2007 to 2014. (B) Mixed-model analysis of variance of the fixed effect of crops and year on dry biomass production after splitting the data set by location

Source	Numerator df	*F*-value	*P*-value
A			
Crop	2	13.17	0.000
Year	7	80.98	0.000
Location	1	21.81	0.000
Block	2	3.72	0.084
Year × Crop	14	1.43	0.218
Year × Location	7	9.29	0.000
Year × Crop × Location	15	1.83	0.172
B			
Casale			
Crop	2	5.70	0.000
Year	7	9.16	0.001
Block	2	10.86	0.000
Year × Crop	14	0.77	0.682
Gariga			
Crop	2	28.44	0.000
Year	7	118.04	0.000
Block	2	4.02	0.118
Year × Crop	13	6.08	0.002

mum yield from the second year (Fig. 1). On average of the first eight-year period, a significant difference in biomass production was found between switchgrass and the two highest yielding crops, miscanthus and giant reed (Fig. 1). The two-way interaction between crops and year was significant in Gariga (Table 2B). While switchgrass and miscanthus reached its maximum biomass yield already in the second year after establishment, giant reed increased its biomass yield until the third year (Fig. 2). After having reached their maximum yield, miscanthus and switchgrass biomass did not vary significantly across years, while biomass fluctuation was observed in giant reed (Fig. 2).

The analysis of the effect of nitrogen on biomass production showed a different response between giant reed and switchgrass (Table 3). In particular, nitrogen fertilization affected significantly switchgrass biomass production that increased by 16% in the fertilized plots, from 12 to 13.9 Mg ha^{-1} yr^{-1} ($P < 0.001$), whereas giant reed biomass production was not significantly affected by nitrogen fertilization.

No significant relationship was found between biomass production and growing degree days$_{10}$ calculated from May to August ($r^2 = 0.01$, $P = 0.68$) and from June to September ($r^2 = 0.01$, $P = 0.78$) in both location. The linear regression performed to test the response of herbaceous biomass production to the water input (irrigation + rainfall) calculated from June to September was found not significant ($r^2 = 0.02$, $P = 0.62$). The relationship between biomass production and summer water input (i.e., the sum of rainfall from June to September and irrigation, when performed) was significant only for miscanthus in Casale ($r^2 = 0.6$, $P < 0.10$). A significant relationship was found between the water input calculated from May to August (adding irrigation, when performed) and biomass production of miscanthus in both locations, and in giant reed in Gariga with and without nitrogen fertilization. The response to the water input calculated from May to August was significant in switchgrass only in the fertilized plots (Table 4).

The annual biomass production was estimated for each woody crop dividing by two the production of a single harvest, which was collected in 2008, 2010, and 2012 in Casale, and in 2008 and 2010, in Gariga, for last harvest in Gariga (2013) biomass yield was divided by

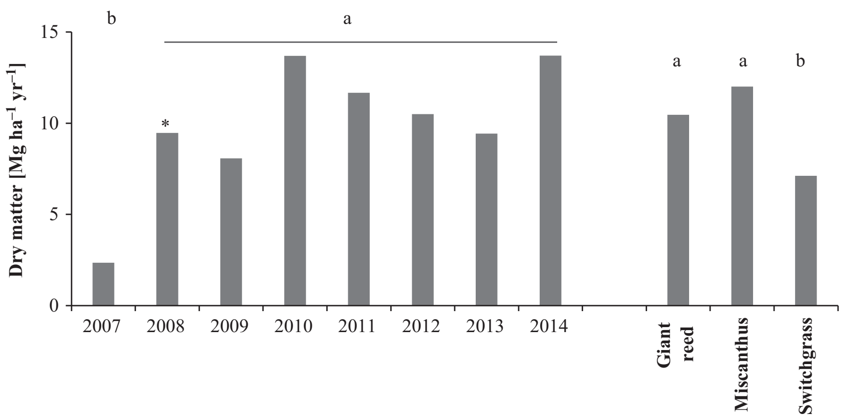

Fig. 1 Biomass production of herbaceous crops observed in Casale from 2007 to 2014 (left) and mean biomass production of giant reed, miscanthus, and switchgrass across years (right). Letters indicate Tukey's least mean significant difference between years (left) and crops (right). *indicates the year of full crops establishment.

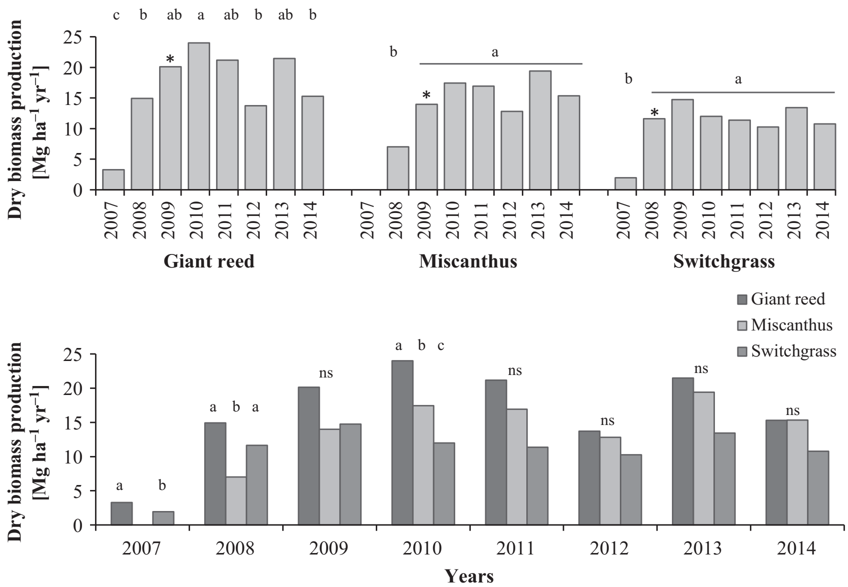

Fig. 2 Mean annual biomass production of giant reed, miscanthus, and switchgrass observed in Gariga from 2007 to 2014 (above) and mean comparison between crops within years (below). Letters indicate Tukey's least mean significant difference between years within crops (above) and crops within year (below). *indicates the year of full crops establishment. "n.s."=not significant.

Table 3 Mixed-model analysis of variance of the fixed effect of nitrogen and year on dry biomass production of giant reed and switchgrass in Gariga from 2008 to 2014

Source	Numerator df	*F*-value	*P*-value
Giant reed			
Year	7	83.42	0.000
Nitrogen	1	0.21	0.655
Block	2	1.61	0.289
Year × Nitrogen	6	1.61	0.265
Switchgrass			
Year	7	149.96	0.000
Nitrogen	1	11.68	0.003
Block	2	1.77	0.351
Year × Nitrogen	6	0.86	0.549

three. The annual biomass production of the woody crops was significantly lower in Casale (7.1 Mg ha^{-1} yr^{-1}) than in Gariga (10 Mg ha^{-1} yr^{-1}, $P < 0.001$). When each location is examined independently, the annual production estimated from the first harvest was lower (4.3 Mg ha^{-1} yr^{-1}) than that from the second and the third harvests (7.7 and 9.2 Mg ha^{-1} yr^{-1}, $P < 0.05$) in Casale; similarly, in Gariga, annual production estimated from the three harvests was 3.3, 12.9, and 12.2 Mg ha^{-1} yr^{-1}, respectively. No significant difference of biomass production among crops was found in Casale, although black locust doubled the annual production of willow in the third harvest (Fig. 3). Apparently, the high variability between blocks in Casale overwhelmed the variability between

Table 4 Linear regression between annual herbaceous production and water input observed in Casale (rainfall + irrigation) and in Gariga (irrigation) from June to August, considering the period after full crop establishment (i.e., 2010–2014)

	Casale Rainfall (May–August) + Irrigation		Gariga Rainfall (May–August)	
Crops	r^2	*b*	r^2	*B*
Giant Reed	0.30 ns		0.63*	52.7
Miscanthus	0.6*	25.4	0.70*	31.3
Switchgrass	0.07 ns		0.40 ns	
Giant Reed 100N			0.80**	74.1
Switchgrass 100N			0.94***	20.8

b is the biomass increasing rate for the unit water input (kg mm^{-1}). 'ns', not significant.
*Significant the 0.10 level.
**Significant the 0.05 level.
***Significant the 0.01 level.

crops (coefficient of variation = 78%, 55%, 1.38%, in poplar, black locust, and willow, respectively). The variability between blocks was likely due to a different content of coarse sand (12%, 59%, and 12% in block 1, 2, and 3, respectively) in the upper 40 cm of soil. In Gariga, the significant interaction between crops and harvest is explained by the lowest biomass production of black locust that was observed in 2010 (second har-

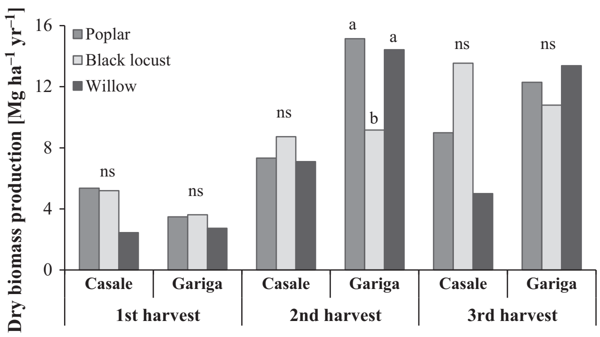

Fig. 3 Annual biomass production of woody crops estimated dividing by two the biomass harvested in 2008 (1st harvest), 2010 (2nd harvest), and 2012 (3rd harvest) in Casale and in 2008 (1st harvest), 2010 (2nd harvest) in Gariga. The annual biomass of woody crops of the third period (3rd harvest) was calculated dividing by three the production harvested in 2013 in Gariga. Letters indicate Tukey's least mean significant difference between years and crops within harvest and location. "n.s."=not significant.

vest). Thus, only the biomass production of black locust increased significantly from the second to the third harvest despite the drought experienced in the summer of 2012 (Table S3).

Comparing the mean annual production of herbaceous and woody crops, no significant difference was found in Casale. In Gariga, giant reed production doubled that of black locust and it was 1.5 times higher than the production of switchgrass, willow, and poplar; miscanthus yielded significantly less than giant reed with a decrease of 26% (Fig. 4).

Biomass quality

The qualitative analysis carried out on dry samples collected in 2013 showed that (i) the cellulose and ash con-

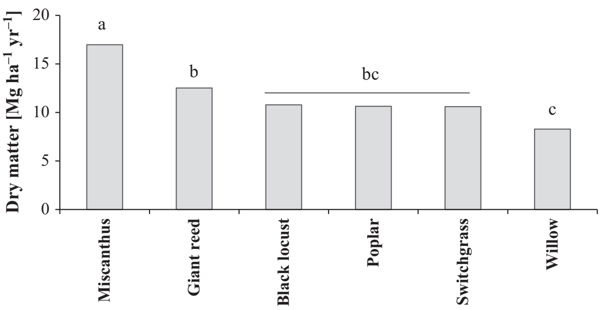

Fig. 4 Mean annual biomass production of herbaceous and woody crops in Gariga from 2007 to 2013. Annual biomass production of woody crops was estimated dividing by two the biomass harvested in 2008 (1st harvest), 2010 (2nd harvest), and by three that harvested in 2013 (3rd harvest). Letters indicate Tukey's least mean significant difference between crops.

tent of miscanthus was comparable to that of the woody crops, (ii) the hemicellulose content of switchgrass was higher than that of the other crops, (iii) the lignin content was lowest in giant reed, and (iv) the ash content was highest in giant reed (Table 5).

The low heating value (LHV) was different between crops ($P < 0.05$). Giant reed LHV was significantly lower than that of the other crops (16.7 MJ kg^{-1}) that was 17.8 MJ kg^{-1} for miscanthus and switchgrass, and on average 18.8 MJ kg^{-1} for the woody crops. The differences in LHV between herbaceous and woody crops were likely due to the different biomass composition, namely lignin content.

Energy balance

A simplified energy balance was calculated to compare NEG and EROI at the farm gate for the six bioenergy crops in both locations. Energy input and output, and in turn NEG and EROI, were different between crops and locations and varied according to biomass production, low heating value (LHV), and energy costs due to planting, and management (Table S2).

In both locations, the energy required for woody crops planting was 1.4 times higher than that of herbaceous crops because of the higher fossil fuel consumption, which was on average 5.8 and 4.7 GJ ha^{-1} yr^{-1} for woody and herbaceous crops, respectively. Among woody crops, black locust required the lowest management energy input because nitrogen fertilization was not carried out. In Casale, the highest cost of woody crops production was required for management operations (mainly irrigation, 79%), while harvest and planting required 13% and 8% of the total energy costs, respectively. Poplar and willow required the highest energy input among the six studied crops in Casale (Table S2). Considering the energy costs associated with herbaceous crops cultivation, the energy required for management was the highest (49.7 GJ ha^{-1}). Giant reed had higher harvest costs than miscanthus and switchgrass due to the higher biomass production.

In Gariga, the highest proportion of energy costs was allocated to harvesting in the unfertilized herbaceous crops (77%) and to management (68%) in the fertilized crops due to the application of nitrogen fertilizer. Among the studied crops in Gariga, the highest energy costs were calculated for fertilized giant reed and switchgrass cultivation.

Direct and indirect energy costs of harvesting were higher in herbaceous than in woody crops (Table S2), as a consequence of the highest harvesting frequency (every year) and biomass production of the herbaceous crops.

Table 5 Composition (%) of the studied crops biomass harvested in 2013 after senescence in Gariga

	F-value	Black locust	Poplar	Willow	Giant Reed	Miscanthus	Switchgrass
Cellulose	23.5***	52.2 a	53.6 a	54.6 a	42.6 b	50.0 a	42.4 b
Hemicellulose	112.3***	18.1 c	18.6 c	19.1 c	28.7 b	30.2 b	36.0 a
Lignin	33.9***	16.9 a	15.9 ab	13.3 abc	7.2 d	11.5 bcd	9.6 cd
Ash	100.8***	1.7 c	2.1 c	2.1 c	7.5 a	1.7 c	2.9 b

*** is significant of 0.01 level.

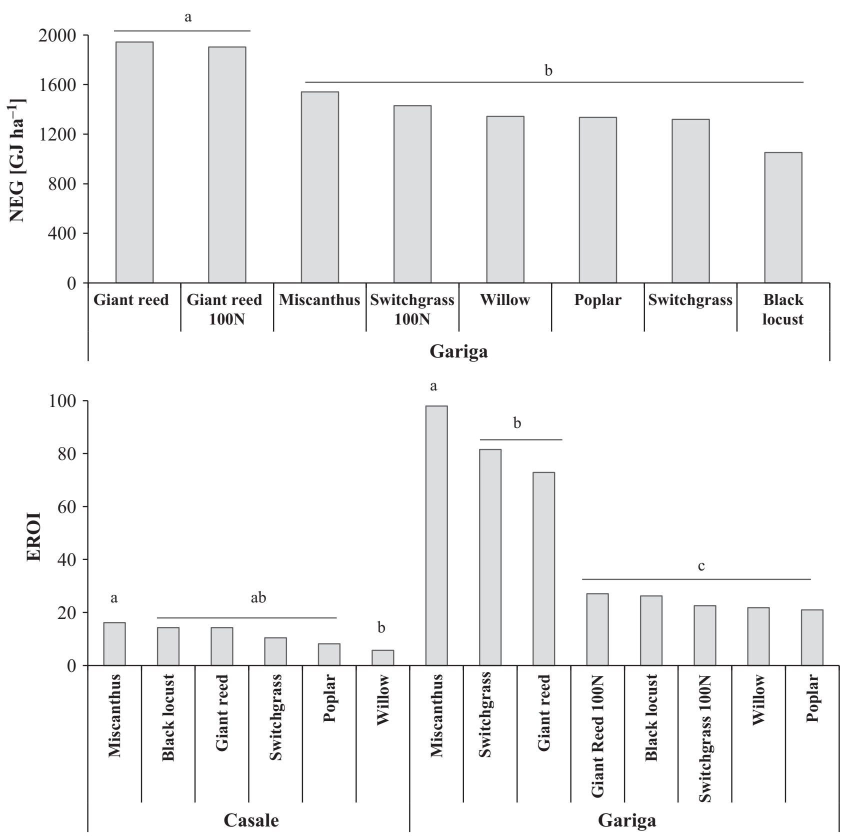

Fig. 5 Net energy yield (output – input, NEY) estimated for the six studied crops considering the cumulative energy input and output from 2007 to 2013 in Gariga (above). Energy Return On Investment (output: input, EROI) estimated for the six studied crops considering the cumulative energy input and output from 2007 to 2012 in Casale and from 2007 to 2013 in Gariga (below). Letters indicate Tukey's least mean significant difference between crops within locations.

Variation in NEG and EROI was mainly driven by the large differences in biomass production between crops and locations (Fig. 5).

With regard to energy output, miscanthus and black locust had the highest value in Casale (Fig. 5). NEG calculated in Casale and Gariga was higher for herbaceous crops (average value 903 and 1627 GJ ha^{-1}, respectively) than for woody crops (727 and 1244 GJ ha^{-1}).

Although differences in NEG between crops were not significant in Casale, the NEG of miscanthus, giant reed, and black locust was 2.3 times higher than that of willow. Average NEG of crops in Casale was 2 times lower than that observed in Gariga where giant reed NEG was 1.5 times higher than that calculated for the other crops. In Gariga, fertilized and unfertilized giant reed had similar energy output (1985 and 1971 GJ ha^{-1}, respectively) and NEG (1943 and 1903 GJ ha^{-1}, respectively) despite the different energy input costs (82 and 28 GJ ha^{-1}, respectively). In switchgrass, nitrogen fertilization induced a significant increase in energy output (from 1333 to 1506 GJ ha^{-1}, respectively) and a not significant increase in NEG (from 1318 to 1430 GJ ha^{-1}, respectively).

As expected, the variation of energy input due to nitrogen fertilization produced a larger variation of EROI than of NEG. In giant reed, when the input increased by 3 times (from 28 to 82 GJ ha^{-1}) due to fertilization, NEG decreased by 2% ($P = 0.99$), whereas EROI by 2.7 times ($P < 0.001$). In switchgrass, when input increased by 5 times (from 15 to 76 GJ ha^{-1}) due to fertilization, NEG increased by 8% ($P = 0.11$), whereas EROI decreased by 3.6 times ($P < 0.001$).

The mean EROI index estimated for Casale was 4 times lower than that of Gariga because of the lower biomass production and the higher energy costs due to irrigation (Table S2). Miscanthus EROI index was the highest both in Casale and in Gariga. Herbaceous crops had higher EROI than woody crops in Gariga, while black locust had a similar EROI index to miscanthus in Casale.

Discussion

In this study, a comparison of biomass production of six perennial bioenergy crops over 8 years in two different locations is presented, and for the first time, a direct comparison between 3 herbaceous and 3 woody biomass crops cultivated in the same experimental conditions is discussed.

On average, herbaceous crops had higher biomass yield than woody crops in both locations. This is in agreement with the ranking of energy crops on the basis of a meta-analysis carried out by Laurent *et al.* (2015) on the yield data from 28 published papers.

The most and the least productive crops were miscanthus and willow in Casale and giant reed and black locust in Gariga (Fig. 4). To compare our results with the data reported in literature, medians of pooled biomass data were plotted over the boxplot chart of the dry biomass yield shown in Laurent *et al.* (2015). The yield levels reported in our study are consistent with those presented in the meta-analysis, with the best agreement being found for miscanthus and switchgrass (Fig. 6), for which a wide set of data from different environments was available. Giant reed, while showing a relatively high yield in both locations, falls within the 1st quartile of literature data (Laurent *et al.*, 2015). This can be explained by the marginal nature of both locations in this study, and by the fact that most of the very high annual yields reported for giant reed (>30 Mg ha^{-1} yr^{-1}) were obtained in the optimal, often irrigated, conditions of the center and south of Italy, where plant growth is extended thanks to the relatively high temperature registered in autumn and winter (Scordia *et al.*, 2014). It is interesting to note (Fig. 6) the very different performances of willow in Gariga, where it ranked across the 2nd and 3rd percentiles, and in Casale, where it equaled the lowest yield reported in literature (Laurent *et al.*, 2015). Willow is well adapted to environments with high water availability and soil with high water holding capacity (Dimitriou *et al.*, 2011); it therefore found suitable conditions in the silty soil of Gariga, where after a slow establishment (1st harvest Fig. 3), it had a relatively high productivity (Abrahamson *et al.*, 2002; DEFRA, 2002). On the contrary, in the sandy soil of Casale, willow had very low yields in the phase of establishment (1st harvest, Fig. 3) and at full production (2nd and 3rd harvests). The marked

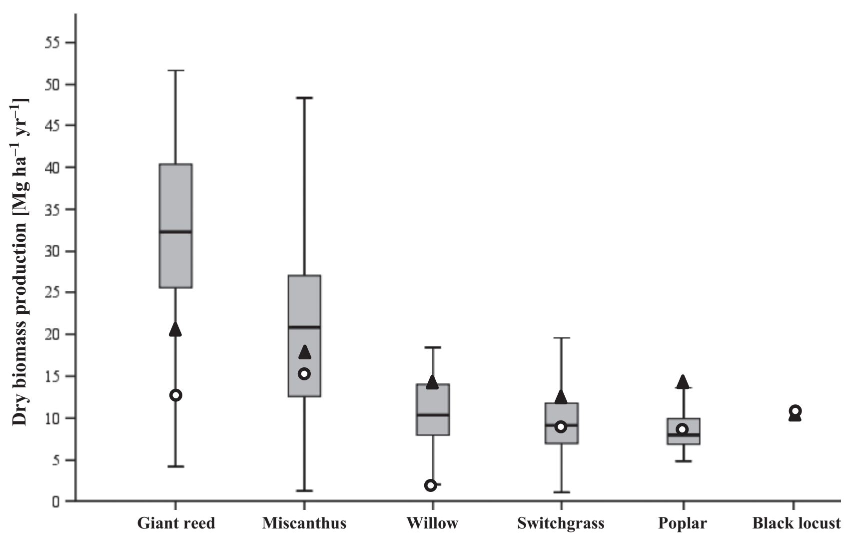

Fig. 6 Comparison between median biomass production of the six crops observed in this study across year (2007-2014) and the boxplot of the dry biomass production reported by Laurent *et al.* (2015) for giant reed, miscanthus, willow, switchgrass, and poplar. White circle and black triangle indicate the median production observed across years in this study in Casale and in Gariga, respectively.

decrease in production at the 3rd harvest was probably a consequence of plant mortality (plant density was 7500 plants ha^{-1} at establishment and reduced to 4500 plants ha^{-1} at the 3rd harvest). Despite the sandy soil and low water availability in Casale, the median value of poplar biomass was very similar to that found in literature (Laurent *et al*., 2015), which confirms the tolerance of poplar genotypes belonging to the *P. deltoides* crops to environments with coarse soils and periods of drought (Bergante *et al*., 2010). In the silty-loam soil of Gariga, poplar found ideal conditions, reaching the highest yields found in literature (Fig. 6).

Limited information on the productivity of black locust as a bioenergy crop is present in literature and no direct comparison with other bioenergy crops is reported; for this reason, black locust was not included in the meta-analysis performed by Laurent *et al*. (2015). Straker *et al*. (2015), in a recent review on black locust as a bioenergy crop, reported an average biomass production of 10 Mg h^{-1} yr^{-1} in Italy, which is in line with the data presented in this study (10.6 Mg h^{-1} yr^{-1}). Interestingly, black locust had a very similar performance in Gariga and Casale, which denotes yield stability. Black locust is considered as being particularly adapted to marginal coarse (light)-textured soil, while it is sensitive to poorly drained or compact plastic soils (Straker *et al*., 2015). The lower yield of black locust in the first period of growth, in comparison with the other woody crops, was probably the consequence of its difficult establishment in the compact silty-loam soil of Gariga. In the 3rd period, when mean summer rainfall (91 mm cumulative rainfall from June to August) was lower than in the previous periods (146 mm 1st period and 120 mm 2nd period), black locust reached the same level of production of poplar and willow (Fig. 3).

It is apparent that water availability (as a function of rainfall/irrigation and soil water holding capacity) was one of the major factors affecting biomass yield in this study and in several others (e.g., Heaton *et al*., 2004a; Arundale *et al*., 2014). However, in this study, it was difficult to find a clear relationship between yield of woody crops and water availability, because plants were harvested every two years (three in the 3rd harvest in Gariga) and any environmental effect was therefore spread over the whole growing period. Moreover, considering that in the 1st period biomass yield was limited due to crop establishment, only data from two harvests could be used to study the relationship between environmental parameters and woody crop yield. For this reason, the relationship between agronomic variables and crop yield is only discussed for the herbaceous crops and more in detail for the trial carried out in Gariga where nitrogen was applied (on giant reed and switchgrass).

Biomass production for none of the herbaceous crops (in Casale or Gariga) was affected by growing degree $days_{10}$, as already shown by Heaton *et al*. (2004a) for miscanthus and switchgrass. On the contrary, a significant relationship was found between water input calculated from May to August and miscanthus biomass production in both locations, and in giant reed and fertilized switchgrass in Gariga (Table 4). Considering the period June–September, when giant reed and miscanthus evapotranspiration is highest (Triana *et al*., 2015), the relationship between biomass production and water input was significant only for miscanthus in Casale. In the fine-textured soil of Gariga, all herbaceous crops were therefore affected by water input more in late spring than in September, at the end of the growing season.

Miscanthus confirmed to have a stronger response to water than switchgrass as previously reported by Heaton *et al*. (2004a). Biomass production of giant reed was affected by water availability only in Gariga; in this site and on the same experiment, the root system of the six studied crops was characterized by Chimento & Amaducci (2015) and giant reed root biomass was 2 times lower than that of switchgrass. It can therefore be assumed that having a lower root biomass was a factor in the higher sensitivity of giant reed to water availability than switchgrass. Switchgrass biomass production was highly positively related to water availability only when nitrogen fertilization occurred; the lack of response to water availability in the nonfertilized plots indicates that nitrogen was the limiting production factor and confirms the sensitivity of switchgrass to nitrogen availability (Table 3) (Heaton *et al*., 2004a). The lack of significant response of giant reed to nitrogen fertilization can be related to the translocation of nutrients from the leaves and the stems to the rhizomes, which takes place in giant reed and miscanthus at the end of the growing season (Heaton *et al*., 2004a; Cosentino *et al*., 2014).

The trend of biomass production in the first 8 years after planting, excluding the initial increase during establishment, did not show any significant decline in either Casale or Gariga for any of the herbaceous crops (Figs 1 and 2). Gauder *et al*. (2012) also reported miscanthus yield variability between years but did not found any decline in a 14-year experiment in Germany. Our result is also in agreement with Lesur *et al*. (2013) who reported maximum yields of miscanthus reached after 6–13 years in long-term experiments in 16 locations in Europe. As reported by Lesur *et al*. (2013), also Clifton-Brown *et al*. (2007) and Christian *et al*. (2008) found a miscanthus yield decline after year 10 and 11, respectively. Conversely, the result obtained in the pre-

sent study in contrast to the data reported by Arundale *et al.* (2014) for miscanthus production in the United States, while it is in agreement with most of the experiments carried out in Europe (Heaton *et al.*, 2004b). As highlighted by Arundale *et al.* (2014), the yield decline of miscanthus after the 5th - 6th year of stand age might be a consequence of the nutrient depletion relative to the very high biomass yield obtained in their trial, in contrast to the lower biomass yields reported in this study.

To support the choice of the most suitable biomass crops for a specific environment and end use destination, besides biomass yield, biomass quality should also be considered. Data on cellulose, hemicellulose, lignin, and ash content presented in this study (Table 5) are relative to one year (2013) and one location (Gariga). The fact that the composition was analyzed in one growing season and in one location does not affect the reliability of the results as reported by Arundale *et al.* (2015), who found that there was minimal variation in the composition of miscanthus samples across location, sampling times, and fertilization treatments. The composition values measured in this study are in agreement with those reported in literature for the herbaceous (Arundale *et al.*, 2015; Mohammed *et al.*, 2015) and for the woody crops (Sannigrahi *et al.*, 2010). This study confirms that the herbaceous crops have a lower quantity of lignin than the woody crops and a higher ash content, in particular for giant reed and switchgrass (Table 5). These characteristics enable herbaceous crops to be suitable also for biochemical transformations and not only for thermochemical conversion, as the woody crops (Monti *et al.*, 2015). Among the herbaceous crops, miscanthus is the most suitable for anaerobic digestion and second-generation biofuel production due to its high cellulose and hemicellulose content (Table 5) (Monti *et al.*, 2015).

Energy balance

The low heating value (LHV) of the six crops considered in this study varied between crops and it was significantly higher in woody than in herbaceous crops. This was due to the biomass composition: Woody crops had a high lignin content that has a higher LHV than cellulose and hemicellulose (Furlan *et al.*, 2013) and a lower ash content (Ciolkosz, 2010). The measured LHV was in line with data reported in literature for both herbaceous (Angelini *et al.*, 2005, 2009; Mantineo *et al.*, 2009) and woody crops (McKendry, 2002; Nassi o Di Nasso *et al.*, 2010; Dillen *et al.*, 2013). In addition, LHV of giant reed and switchgrass was not affected by nitrogen fertilization; Ercoli *et al.* (1999) found the same trend in miscanthus.

The application of nitrogen fertilizer represented 70% of total energy costs in Gariga, in accordance with results from Angelini *et al.* (2005) relative to giant reed cultivation in a 6-year experiment in central Italy. Similarly, irrigation represented a high percentage of total energy costs (70%) in Casale, which is in agreement with Mantineo *et al.* (2009) who reported that irrigation costs were the highest in cultivation of giant reed, miscanthus, and *Cynara cardunculus* in a 5-year experiment in southern Italy.

The NEG of giant reed and miscanthus in this study was lower than those reported by Angelini *et al.* (2009) in central Italy, and the NEG of poplar was lower than that reported by Nassi o Di Nasso *et al.* (2010) in a 12-year short-rotation coppice poplar in central Italy. These differences are mainly due the lower biomass yields obtained in our trial. For switchgrass, however, NEG values were similar to those found by Monti *et al.* (2009b) who reported a mean annual NEG of 200 GJ ha^{-1} yr^{-1} for switchgrass fertilized with 200 kg ha^{-1} yr^{-1} of nitrogen.

In this study, the EROI of black locust was higher than that of the other woody crops (Fig. 5); in Gariga for the lack of nitrogen fertilization and the consequent lowest energy cost, in Casale, for the highest biomass yield. NEG of black locust was higher than that reported by Stolarski *et al.* (2015) for a SRC plantation of 4 years in Poland. Manzone *et al.* (2015) reported a NEG of 190 GJ ha^{-1} yr^{-1} in a black locust 7-year experiment in Casale, which is higher than that reported in this study in the same environment (144 GJ ha^{-1} yr^{-1}), and in Gariga (167 GJ ha^{-1} yr^{-1}). This is a consequence of the higher biomass production (10 Mg ha^{-1} yr^{-1}) and higher LHV (19 MJ kg^{-1}) reported by Manzone *et al.* (2015) (average biomass production 9 Mg ha^{-1} yr^{-1} and LHV 18 MJ kg^{-1} in this study).

EROI values calculated for Gariga were in line with those found by other authors for unfertilized giant reed (Angelini *et al.*, 2005), fertilized giant reed and switchgrass (Monti *et al.*, 2009b), and woody crops (Djomo *et al.*, 2011; Dillen *et al.*, 2013). EROI of miscanthus (98) and unfertilized switchgrass (82) grown in Gariga was higher than those reported by Angelini *et al.* (2009) and Monti *et al.* (2009b), mainly as a consequence of different nitrogen inputs. In Casale, woody and herbaceous crops had EROI values lower than those reported in literature (Angelini *et al.*, 2009; Monti *et al.*, 2009b; Djomo *et al.*, 2011; Dillen *et al.*, 2013), because of lower biomass production and higher energy input due to the irrigation. Across locations, black locust had a mean EROI of 20 that is in agreement with that reported by Manzone *et al.* (2015). Several authors also found that black locust has a high energy efficiency (González-García *et al.*, 2011; González-García *et al.*, 2012; Manzone *et al.*, 2015).

In conclusion, herbaceous crops in this study had the highest ranking for bioenergy production due to their high biomass yield, high NEG, and biomass quality that renders them suitable to both biochemical and thermochemical conversion. Among the woody crops, black locust biomass production was comparable to that of the best herbaceous crops in the water-limited environment of Casale, while it proved less suitable for the fine-textured and fine-compacted soil of Gariga. Nitrogen fertilization of giant reed is not recommended as it did not affect biomass or energy production, and as a consequence, it significantly reduced energy efficiency (EROI). In switchgrass, however, nitrogen fertilization significantly increased biomass production and the response of this crop to water availability, making it a favorable option when only biomass production is a target.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mean annual biomass production of giant reed, miscanthus, and switchgrass observed in Casale from 2007 to 2014.
Table S1. Main characteristics of soil layers in Casale and Gariga.
Table S2. Energy costs [GJ ha^{-1}] of the studied species cultivation in Casale (2007–2012) and in Gariga (2007–2013).
Table S3. Monthly rainfall and mean air temperature measured in Casale and in Gariga from 2007 to 2014.